NOV 30 2005

This report is required by law (? USC 21 43) Failure to report according to the regulations can result in an order to cease and desist and to be subject lo penalties as provided for in Section 2150

Set reverse side for additionalinformation

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

1. REGISTRATION NO. 58-R-0003 cust.#-859

FORM APPROVED OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY (Name and Address, as registered with USDA include Zip Code)

University of Florida
PO Box 115500
Gainesville, FL 32611
(352) 392-9271

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS (Sites)

See attached listing.

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS FORM 7023A)

A. Animals Covered By The Anima Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress. or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used	E. Number of animals upon which teaching experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in those animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO OF ANIMALS (Cols. C + D + E)
4. Dogs		30	83		113
5. Cats		33	473		506
6. Guinea Pigs		50	143		193
7. Hamsters		0	0		0
8. Rabbits		14	110	160	284
9. Non-human Primates			111		111
10. Sheep			134		134
11., Pigs			48		48
12. Other Farm Animals					
Horses		108	29		137
13 Other Animals					
Chinchillas			92		92
Deermice		120	9		129
Singing Mice		58			58

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary **of all such exceptions is attached lo this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarianfor this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional Official)

I certify that the above is true, correct, and complete (7 U.S.C. Section 2143).

SIGNATURE OF CEO OR INSTITUTIONAL OFFICIAL	NAME & TITLE OF CEO OR INSTITUTIONAL OFFICIAL *(Type or Print)*	DATE SIGNED
(b)(6), (b)(7)c		11/23/05

AP (Replaces VS FORM 18-23 (OCT 88) which is obsolete

(AUG 91)

RCA

Attachment 1A

This report is required by law (7 USC 2143) Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150

Set reverse side for additional information

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Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

CONTINUATION SHEET FOR ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

1. REGISTRATION NO.
58-R-0003 cust.# - 859

FORM APPROVED
OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY (Name and Address, as registered with USDA include Zip Code)
University of Florida
PO Box 115500
Gainesville, FL 32611
(352) 392-9271

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS FORM 7023A)

A. Animals Covered By The Anima Welfare Regulations 12. &/OR 13. Other *(List by species)*	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used	E. Number of animals upon which teaching experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in those animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO OF ANIMALS (Cols. C + D + E)
Cattle		41	7		48
Voles		129	60		189

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary **of all such exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional Official)
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143).

SIGNATURE OF CEO OR INSTITUTIONAL OFFICIAL	NAME & TITLE OF CEO OR INSTITUTIONAL OFFICIAL *(Type or Print)*	DATE SIGNED
(b)(6), (b)(7)c		11/23/05

APHIS FORM 7023A
(AUG 91)

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Facilities under the care of Animal Care Services housing USDA-covered species:

University of Florida P.O. Box 100006
Gainesville, FL 32610 (352) 392-2978

1.

2.

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5.

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Attachment 2

Column E Explanation Form

This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

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1. **Registration Number:** 58-R-0003

2. **Number of animals used under Column E conditions in this study.** 0

3. **Species (common name) of animals used in this study.** Rabbits

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**

   Title: D172; Gene regulation of mammalian DNA viruses
   Procedure: Please see attached.

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.**

   Please see attached.

Information below will NOT be forwarded to USDA as part of the Annual Report

IC_____ **ASP Number**_____ **ASP Title**_____

Signature of Principal Investigator_____**Date** _____

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Procedures for treatment of the rabbits will be as follows:
Animals will be restrained in a rabbit restrainer (provided by ACS) and the area over and around the ear vein will be washed with ethanol. Using a 25-gauge needle (or catheter), 1-2 ml of anti-viral treatment will be administered slowly into the ear vein. After removal of the needle, gentle pressure will be applied to the ear to minimize bleeding. If the treatment dose is to be administered at Day 1, 2, or 4 post-infection, a second injection will be given in the other ear vein using the procedure listed above.
Procedure for Administration of Virus:
The rabbits will be gently restrained with a towel. A 10 cm area of skin on the lateral aspect of each flank will be shaved and washed with ethanol. 100 microliters of buffer and virus will be injected intradermally at the center of the shaved area on lateral aspect of both thighs using a sterile 25 gauge needle.
Some of these animals will become severely ill from the virus, and will be allowed to reach an endpoint before euthanasia so that treatment efficacy can be scientifically evaluated.
Procedure for microchip placement:
A sterile Implantable Programmable Temperature Transponder chip (Biomedic Data Systems Inc.) will be injected subcutaneously under the skin at the nape of the neck (dorsal cervical region). The chip transmits data for animal identification and body temperature. The rabbits will be gently restrained with a towel. The skin over the nape of the neck will be gently tented away from the back muscles. The tented fur at the nape of the neck will be washed with ethanol. A 2.2 by 14 mm transponder chip will be inserted under the skin by injection via the sterile 14 gauge needle that houses the chip (provided by Biomedic Data Systems Inc.) The skin will be released and physically examined to ensure the chip is placed under the skin and there is no damage to the skin.
Routine Physical examination:
A routine physical exam (abdominal palpation, auscultation of heart and lungs, assessment of attitude and hydration, etc.) will be done daily.
Collection of blood:
Blood collection will be a non-survival procedure in this experiment. For collection of 10 ml blood, 50mg/kg ketatmine and 10 mg/kg xylazine will be injected intramuscularly into the biceps femoris muscle. Blood will be collected by cardiac puncture using a sterile 22 gauge 1½ inch needle attached to a 12 cc syringe. 100-150 mg/kg body weight of pentobarbital will be injected into the heart after blood has been collected to induce euthanasia.
Rabbits are the best animal models for human smallpox because the pathology is much more like that found in the human disease. Other models (mice) also require a higher dose of virus, which makes the efficacy of new treatment difficult to ascertain.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Rabbitpox is a lethal infection in rabbits. Following injection of the poxvirus, these animals will be given different treatments at various dosages to determine the effect of the treatment schemes. Therefore, providing these animals with any other form of anesthesia, analgesia or treatment will not be appropriate. The animals will be closely observed, up to four times a day for signs of severe disease (as indicated by decreased respiratory rates, dyspnea, open-mouth breathing, severe lung sounds, temperature equal to or exceeding 106o F, anorexia, dehydration, and/or morbidity) at which point the animals will be euthanized according to the AVMA Panel on Euthanasia.

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Attachment 2

Column E Explanation Form

This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

---

1. **Registration Number:** 58-R-0003

2. **Number of animals used under Column E conditions in this study.** 122

3. **Species (common name) of animals used in this study.** Rabbits

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**

   Title: D290; Gene regulation of mammalian DNA viruses
   Procedure: Please see attached.

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.**

   Please see attached.

Information below will NOT be forwarded to USDA as part of the Annual Report

IC_____ **ASP Number**_____ **ASP Title**_____

Signature of Principal Investigator_____**Date** _____

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Procedures for restraint of the rabbits will be as follows:
Animals will be restrained in a rabbit restrainer (provided by ACS) or by using a clean towel with minimal restraint by an animal handler. The rabbits will be restrained using a rabbit restrainer or with a towel and both hind flanks will be shaved. The rabbits will be gently restrained with a towel and then injected interdermally with 100ul of buffer and the virus injected intradermally in one or two sites using a 25 gauge needle in one or both hind flanks concurrently in Experiment 1 and 3, with animals in Experiment 2 receiving 100ul of buffer and the virus injected intradermally in one to five sites concurrently using a 25 gauge needle in one or both hind flanks. A sterile Implantable Programmable Temperature Transponder chip (Biomedic Data Systems, Inc.) will be injected subcutaneously at the nape of the neck (dorsal cervical region). The chip transmits data for animal identification and body temperature. The rabbits will be gently restrained with a towel. The skin over the nape of the neck will be gently tented away from the back muscles. The tented fur at the nape of the neck will be washed with ethanol. A 2.2 by 14 mm transponder chip will be inserted under the skin by injection via the sterile 14 gauge needle that houses the chip. The skin will be released and physically examined to ensure the chip is placed under the skin and that there is no damage to the skin. A routine physical exam (abdominal palpitation, auscultation of the heart and lungs, assessment of attitude and hydration, etc.) will be performed daily.
Collection of Blood (Survival):
Rabbits are minimally restrained. The animal is shaved and the skin is cleaned with 70% ethanol. 1-2ml of blood is collected from the lateral saphenous vein with a 25 gauge needle every 2-3 days after virus administration. For collection of 10ml blood, 50mg/kg ketamine and 10mg/kg xylazine will be injected into intramuscularly into the biceps femoris muscle or intraperitoneally. Blood will be collected by cardiac puncture using a sterile 22 gauge 1 ½ inch needle attached to a 12cc syringe. 100-150mg/kg body weight of pentobarbital will be injected into the heart after blood has been collected to induce euthanasia.
Some of these animals will become severely ill from the virus and will be allowed to reach an endpoint before euthanasia so that treatment efficacy can be scientifically evaluated.
Necropsy and Tissue collection:
Upon euthanasia with pentobarbital and the confirmation of successful euthanasia, as determined by the absence of heartbeat and respiration, a necropsy of the animal will be performed. Tissue samples from all the organs, as well as lesions on the dermal phase of the animal, will be collected for virus isolation and characterization.
The differential pathogenesis caused by RPV and VV is not recapitulated in rodents, thus rabbits are the model system chosen. A second experimental advantage of Rabbitpox Virus (RPV) is that the disease is quite similar to that caused by smallpox in humans. Hence, the types of genes we identify, which are responsible for acute disease in rabbits, should be quite relevant to smallpox in humans.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Rabbitpox is a lethal infection in rabbits. Analgesics are not indicated in these studies because their affects on the inflammatory and immune responses would hinder the distinctions observed in the two viral diseases. It is critical that the rabbitpox and vaccinia viral diseases be distinguishable. For the screening of recombinant viruses it is also critical for the disease pathways be uninhibited for the detection of virulent viruses.

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Attachment 2

Column E Explanation Form

This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

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1. **Registration Number:** 58-R-0003
2. **Number of animals used under Column E conditions in this study.** 18
3. **Species (common name) of animals used in this study.** Rabbits
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**

   Title: D878; Gene regulation of mammalian DNA viruses
   Procedure: Please see attached.

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.**

   Please see attached.

Information below will NOT be forwarded to USDA as part of the Annual Report

IC_____ **ASP Number**_____ **ASP Title**_____

Signature of Principal Investigator_____**Date** _____

D878; *Gene regulation of mammalian DNA viruses #1 – myxoma virus in rabbits #2 – cowpox virus in mice*

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Rabbits and mice are the appropriate species. Rabbits are the natural host for myxoma virus, and its pathogenesis has been well described. The studies proposed are an extension of earlier studies with wild-type and recombinant myxoma virus (IACUC #3071 and B-381), and therefore, rely extensively on an established database with this model.

Mice are proposed for the studies with cowpox. We are proposing to explore the early innate and acquired immune responses to these viruses when intratracheally instilled. The viruses infect murine tissues, and the reagents to assess the magnitude and the nature of the immune response are available in mice, but not in rabbits.

Thus, the studies proposed require both species; the rabbits will be used primarily to explore the pathological responses, whereas the mice will be used to explore the immunological basis for these responses.

Animals will not undergo any operation until they have adjusted to the room for at least three days. SOP #0092 and 0093 describe the surgical procedures being preformed. Briefly, after an adequate plane of anesthesia has been obtained (failure to respond to being placed in the supine position, failure to respond to a toe pinch) the animals are placed supine and an area at the ventral surface of the neck is surgically prepared. Using sharp sterile iris scissors, a 0.5 cm incision is made on medial aspect of the throat. A temperature probe is placed subcutaneously and the trachea is exposed by blunt dissection. By visual inspection, a 30 gauge need is passed into the trachea and 32 microliters of fluid containing the virus is directly instilled. The surgical wound is closed with a sterile surgical clip or skin glue and the animal is allowed to awaken while resting on a warming blanket.

Rabbits: Intradermal administration of the virus will produce both a primary lesion at the site of administration, as well as systemic disease. The skin lesions are not treated. Death from the viral infection in the wild, and from experimental infection, is usually a direct result of respiratory failure, and occurs between days 8 and 14. The animals are monitored daily for the first eight days, and body weights are measured. After the first eight days, the animals are monitored three times between the hours of 8 am and 8 pm and once at night (approximately every 6 hours) up to day 14. Although these animals will develop significant respiratory distress, death is not a desired outcome, and animals will be euthanized when evidence of respiratory distress is present. Respiratory and heart rate are evaluated by the veterinarian (Dr. MacNeill). A physical exam (including core temperature and examination of all major organ systems) is performed daily, and the size of the primary lesions is measured. If any animal has frank lung sounds, a markedly reduced respiratory rate, severe respiratory effort or if open mouth breathing is present, the animal will be euthanized.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Analgesics are not indicated in these studies because of their affects on the inflammatory and immune responses being evaluated. Analgesic agents can significantly alter inflammatory processes. Since we will be measuring chemokine and cytokine levels, we cannot alter inflammatory processes in these animals.

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Attachment 2

Column E Explanation Form

This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

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1. **Registration Number:** 58-R-0003
2. **Number of animals used under Column E conditions in this study.** 20
3. **Species (common name) of animals used in this study.** Rabbits
4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**

   Title: D968; Antiviral activity of lipid-conjugated nucleoside analogs against orthopoxviruses
   Procedure: Please see attached.

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.**

   Please see attached.

Information below will NOT be forwarded to USDA as part of the Annual Report

IC_____ **ASP Number**_____ **ASP Title**_____

Signature of Principal Investigator_____**Date** _____

D968; *Antiviral activity of lipid-conjugated nucleoside analogs against orthopoxviruses*

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Rabbits infected with rabbitpox virus are a unique animal model for smallpox studies because the disease closely mimics the pathology of the human disease, and because rabbitpox virus can be spread between animals in the absence of mechanical or insect vectors. In addition, federal regulations require the use of 2 animal species when using animal data to establish efficacy for human drugs.

Procedures for restraint of the rabbits will be as follows:

Animals will be restrained in a rabbit restrainer (provided by ACS) or by using a clean towel with minimal restraint by an animal handler.

Procedure for Administration of Virus:

The rabbits will be restrained using a rabbit restrainer or with a towel and both hind flanks will be shaved. The rabbits will be gently restrained with a towel and then injected intradermally with 100ul of buffer and the virus injected intradermally in one or two sites using a 25 gauge needle in one or both hind flanks.

Procedure for microchip placement:

This procedure does not cause excessive pain to the animal as the chip is placed just under the skin. The benefits of placing the chips into the animals and having them experience a momentary needle stick is far less stress than the alternative which is a rectal temperature taken multiple times within a day. A sterile Implantable Programmable Temperature Transponder chip (Biomedic Data Systems, Inc.) will be injected subcutaneously at the nape of the neck (dorsal cervical region). The chip transmits data for animal identification and body temperature. The rabbits will be gently restrained with a towel. The skin over the nape of the neck will be gently tented away from the back muscles. The tented fur at the nape of the neck will be washed with ethanol. A 2.2 by 14 mm transponder chip will be inserted under the skin by injection via the sterile 14 gauge needle that houses the chip. The skin will be released and physically examined to ensure the chip is placed under the skin and that there is no damage to the skin.

Routine Physical Exam:

A routine physical exam (abdominal palpitation, auscultation of the heart and lungs, assessment of attitude and hydration, etc.) will be performed daily.

Collection of Blood (Survival):

Rabbits are minimally restrained. Approximately an area of 4 inches by 4 inches of the hind flank of the animal is shaved and the skin is cleaned with 70% ethanol. 1-2ml of blood is collected from the lateral saphenous or ear vein with a 25 gauge needle daily. Kidney parameters will not be tested as CDV has not been observed to be nephrotoxic at the dose that is proposed. There has been no nephrotoxocity observed in animals (rabbits as well as mice) given LIP-CDV.

Collection of Blood (Non-survival):

For collection of 10ml blood, 50mg/kg ketamine and 10mg/kg xylazine will be injected intramuscularly into the biceps femoris muscle or intraperitoneally. Blood will be collected by cardiac puncture using a sterile 22 gauge 1 ½ inch needle attached to a 12cc syringe. 100-150mg/kg body weight of pentobarbital will be injected into the heart after blood has been collected to induce euthanasia.

Necropsy and Tissue collection:

Upon euthanasia with pentobarbital and the confirmation of successful euthanasia, as determined by the absence of heart beat and respiration, a necropsy of the animal will be performed. Tissue samples from all the organs, as well as lesions on the dermal phase of the animal, will be collected for virus isolation and characterization.

Administration of Cidofovir:

Cidofovir will be administered in an ear vein dosed at 5-20 mg/kg.

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Administration of LIP-CDV:
The oral LIP-CDV will be administered in a liquid form sweetened with sugar water at a dose of 5-20 mg/kg/day with the animals receiving a total volume of no more than 10ml at a given dosing event.

The intradermal administration of rabbitpox virus will produce both a primary lesion at the site of administration as well as systemic disease. (Bedson, H.S. and Duckworth, M.J. (1963). J.Pathol.Bacteriol. 85, 1-20.) The skin lesions are not treated. Death from the rabbitpox infection in the wild, and from experimental infection, is usually a direct result of respiratory failure, and occurs between days 8 to 14. The animals are monitored daily for the first eight days and body weights measured. After the first eight days, the animals are monitored three times between the hours of 8 am and 8 pm and once at night (approximately every 6 hours) up to day 14. A set of criteria for scoring the level of disease in the animals has been created and is attached. Although these animals will develop significant respiratory distress, death is not a desired outcome and animals will be euthanized when evidence of respiratory distress is present. A physical exam (including respiratory and heart rates, core temperature, and examination of all major organ systems) is performed daily, and the size of the primary lesions are measured. Any animal with frank lung sounds, markedly reduced respiratory rate, open mouth breathing, temperature equal to or exceeding 106°F, anorexia, dehydration, and/or morbidity will be euthanized according to the AVMA Panel on Euthanasia. The symptoms of the disease are not predictable enough to end the studies early. Some animals may develop increased lung sounds that clear within 24-48 hours and the animal make a full recovery. We also have an idea of how the drugs will influence the disease progression, but we must follow the disease in the animals until the appropriate endpoint, which is respiratory distress.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Rabbitpox is a lethal infection in rabbits. Analgesics are not indicated in these studies because their affects would make it difficult to identify any adverse effects caused by drug treatment. It is critical to be able to identify any potential drug related toxicities during the course of these experiments. Analgesics are not indicated in these studies because of their affects on the inflammatory and immune responses that could possibly hinder the typical clinical observations previously documented in rabbits infected with RPV. It is critical that the rabbitpox viral disease be observed unhindered.